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Fukuda et al.

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[54] **DNA FRAGMENT COMPRISING A GENE ENCODING ETHYLENE FORMING ENZYME OF BACTERIA AND THE USE THEREOF**

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[30] **Foreign Application Priority Data**

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[51] **Int. Cl.**[6] **C12N 1/20**; C12N 15/63; C07H 21/04

[52] **U.S. Cl.** **435/252.33**; 435/320.1; 536/23.2

[58] **Field of Search** 435/252.3, 167, 435/252.33, 320.1; 536/23.2

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,877,730 10/1989 Urushizaki et al. 435/132

OTHER PUBLICATIONS

Nagahama, K. et al. (1992) "Classification of ethylene—producing bacteria in terms of biosynthetic pathways to ethylene" *J. Ferment. Bioeng.* 73(1):1–5.

Fukuda et al., *Biochemical and Biophysical Research Communications, vol. 188, No. 2, Oct. 30, 1992, 826–832.*

Nagahama et al., *Journal of General Microbiology,* vol. 137, 1991, 2281–2286.

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Attorney, Agent, or Firm—Bacon & Thomas

[57] **ABSTRACT**

Bacteria such as *E. coli* which are easy in manipulation are transformed by the gene encoding a ethylene-forming enzyme, and ethylene is formed by the transformants.

7 Claims, 2 Drawing Sheets

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                10        20        30        40        50
GCGACCGCGTGTCAGCAACGACAATCCTTACTCGGAGTCGCTGTTCAGGACACTGAAGT

60        70        80        90        100       110
ACTGCCCGCAATGGCCGCAGGATGGGTTTGCCAGTCTTGACGCGGCACGCTCTGGGTGAG

120       130       140       150       160       170
GGATTTCATGCGTTGGTACAACAATGATCACCGGCACAGCCGAATCCGCTTCGTGACACC

180       190       200       210       220       230
GGCTGAGCGGCATCGAGGGCTGGATCATCAGATCCTGGCCAAGCGACATGAGCTGTACGA

240       250       260       270       280       290
GCTAGCCAGAGAGAAAAGGCCGGAGCGGTGGTCGAGGGAGACACGCAACTGGGAACCGAT

300       310       320       330       340       350
CGGCACCGTGCTGTTAAACCCGGATCGAGAGCAAGACGTTGAGAAAAAAGCAGCATAGTT

360       370       380       390       400       410
AGACGGTTGACGCGACAACTACCTTGAAAAACGCCGGCAGGGACGCTCATGATTCATGCT

420       430       440       450       460       470
CCAAGCAGGTGGGGTGTTTTTCCGTCGCTTGGACTTTGTTCTCCCGACGTCGTTTGGAAT

480       490       500       510       520       530
GAGCATCCGTCCCTTTATATGGATAAAGAAGAGACTAGCATGACCAACCTACAGACTTTC
                                          M   T   N   L   Q   T   F

540       550       560       570       580       590
GAGTTGCCTACCGAGGTAACCGGCTGCGCCGCCGATATCTCATTGGGAAGGGCGCTGATC
E   L   P   T   E   V   T   G   C   A   A   D   I   S   L   G   R   A   L   I

600       610       620       630       640       650
CAAGCCTGGCAAAAAGATGGCATTTTTCAGATCAAGACCGATAGTGAGCAGGATCGCAAA
Q   A   W   Q   K   D   G   I   F   Q   I   K   T   D   S   E   Q   D   R   K

660       670       680       690       700       710
ACGCAGGAAGCAATGGCTGCTAGCAAGCAGTTTTGCAAGGAACCGCTGACTTTTAAGAGT
T   Q   E   A   M   A   A   S   K   Q   F   C   K   E   P   L   T   F   K   S

720       730       740       750       760       770
AGCTGCGTTAGCGATCTGACCTACAGCGGCTATGTTGCGTCAGGCGAGGAAGTCACAGCT
S   C   V   S   D   L   T   Y   S   G   Y   V   A   S   G   E   E   V   T   A

780       790       800       810       820       830
GGTAAACCTGATTTCCCTGAAATCTTCACTGTCTGCAAGGACTTGTCGGTAGGCGATCAG
G   K   P   D   F   P   E   I   F   T   V   C   K   D   L   S   V   G   D   Q

840       850       860       870       880       890
CGTGTAAAAGCCGGCTGGCCTTGCCATGGTCCGGTGCCATGGCCAAATAACACCTATCAG
R   V   K   A   G   W   P   C   H   G   P   V   P   W   P   N   N   T   Y   Q

900       910       920       930       940       950
AAAAGCATGAAGACCTTCATGGAAGAGCTGGGTTTAGCGGGCGAACGGTTGCTCAAACTG
K   S   M   K   T   F   M   E   E   L   G   L   A   G   E   R   L   L   K   L
```

FIG. 1

960 970 980 990 1000 1010
ACAGCGCTCGGCTTTGAACTACCCATCAACACGTTCACCGACTTAACTCGCGATGGTTGG
T A L G F E L P I N T F T D L T R D G W

1020 1030 1040 1050 1060 1070
CACCACATGCGTGTATTACGCTTCCCGCCCCAAACATCCACGCTGTCCCGTGGAATTGGT
H H M R V L R F P P Q T S T L S R G I G

1080 1090 1100 1110 1120 1130
GCGCACACTGACTATGGGTTGTTGGTAATTGCCGCTCAGGACGATGTTGGTGGCTTATAT
A H T D Y G L L V I A A Q D D V G G L Y

1140 1150 1160 1170 1180 1190
ATTCGCCCTCCAGTCGAGGGAGAGAAGCGTAATCGTAACTGGTTGCCTGGTGAGAGCTCA
I R P P V E G E K R N R N W L P G E S S

1200 1210 1220 1230 1240 1250
GCAGGCATGTTTGAGCACGATGAACCTTGGACCTTCGTGACGCCCACCCCAGGCGTGTGG
A G M F E H D E P W T F V T P T P G V W

1260 1274 1280 1290 1300 1310
ACAGTTTTCCCAGGTGATATCTTGCAGTTCATGACCGGCGGCCAGCTGCTTTCCACTCCG
T V F P G D I L Q F M T G G Q L L S T P

1320 1330 1340 1350 1360 1370
CACAAGGTTAAGCTCAATACCCGCGAACGTTTCGCCTGCGCTTATTTTCATGAGCCTAAT
H K V K L N T R E R F A C A Y F H E P N

1380 1390 1400 1410 1420 1430
TTTGAAGCATCCGCCTATCCGTTGTTCGAGCCCAGCGCCAATGAGCGTATTCATTATGGT
F E A S A Y P L F E P S A N E R I H Y G

1440 1450 1460 1470 1480 1490
GAGCACTTTACCAACATGTTTATGCGTTGCTATCCAGATCGGATCACCACCCAGAGGATC
E H F T N M F M R C Y P D R I T T Q R I

1500 1510 1520 1530 1540 1550
AACAAGGAGAATCGCCTGGCGCACTTGGAGGACTTGAAGAAGTATTCGGACACCCGCGCG
N K E N R L A H L E D L K K Y S D T R A

1560 1570 1580 1598 1600 1610
ACAGGCTCATGAGTCGACACCCTGCCCCGGTGCTGCCGGACAGGGGCCTTATCGTTACTGG
T G S

1620 1630 1640 1650 1660 1670
TGACTAATAATTGGCATATCAATGTCCACTCAGCACCCAGATCTTATGATCTGGGTGCTG

1680 1690 1700 1710 1720 1730
AGTGGAGCAATGTAACCATTATGCTGAGCGTTCATGCATAGGAATTTCAATAATTCCTAT

1740 1750 1760 1770 1780 1790
ACAAGGCAATCCGCCGAAAAAGGTCCCCTCGGCGTGATGCCAACGTGGCGTCGATGTCGG

1800
CAAAAAGCTT

FIG. 2

DNA FRAGMENT COMPRISING A GENE ENCODING ETHYLENE FORMING ENZYME OF BACTERIA AND THE USE THEREOF

TECHNICAL FIELD

This invention relates to a DNA fragment comprising a gene encoding an ethylene-forming enzyme of bacteria, a vector including the DNA fragment, transformants transformed with the vector, and a method for producing ethylene using the transformants.

BACKGROUND ART

While ethylene is produced from crude petroleum or natural gas, it has long been known that ethylene could also be formed by plants and microorganisms.

Chou et al. reported that α-ketoglutaric acid(α-KG) or L-glutamic acid(Glu) is a precursor of ethylene in *Penicillium digitatum*, a species of fungi (Arch. Biochem. Biophys., 157, 73, 1973), and Goto et al. reported that α-KG serves as a substrate in the ethylene forming system in cell-free extracts of *Pseudomonas syringae* which is one of the species of pathogenic bacteria for plants (Plant Cell Physiol., 28, 405, 1987).

Primrose et al. reported that, in the ethylene-forming system in cell-free extracts of *Escherichia coli,* 2-keto-4-methylthiobutyric acid(KMBA) which is a metabolic intermediate of L-methionine(Met) is a precursor of ethylene biosynthesis (J. Gen. Microbiol.,98,519, 1977). In all these reports, however, the true substrates and biosynthetic pathway of ethylene-forming reaction were not established because materials such as cultured cells of bacteria or their cell free extracts, which are supposed to contain a lot of impurities, were used in the experiments.

To make clear the pathway of the ethylene biosynthesis caused by the bacteria and the ethylene-forming enzyme reaction, the present inventors purified the enzyme catalyzing the ethylene formation to electrophoretically homogeneous state. The results revealed that the ethylene-forming reaction via KMBA is really radical reactions in which active oxygen is concerned (Fukuda et al., FEMS Microbiol. Lett., 60, 107, 1989). We have also investigated the ethylene-forming enzyme of *Penicillium digitatum* IFO 9372 via a α-KG and its enzymatic reaction (Fukuda et al., FEMS Microbiol.lett., 59, 1989), and the ethylene-forming enzyme of *Pseudomonas syringae* pv. phaseolicola PK2 via α-KG and its characteristics (Nagahama, Fukuda et al., J. Gen. Microbiol., 137, 2228, 1991).

Although the enzyme catalyzing the ethylene biosynthesis by bacteria was identified as well as the characteristics of the enzyme, by the present inventors as described above, the ability of ethylene formation in these ethylene-forming bacteria was not adequately sufficient.

For the purpose of fundamental breeding improvement of the ethylene-forming bacteria through a gene manipulation technique, *Pseudomonas syringae* pv. phaseolicola PK2 was selected for the object and its DNA sequence encoding the ethylene-forming enzyme was analyzed, thereby completing this invention.

DISCLOSURE OF INVENTION

To achieve the above described object, this invention is characterized in that a gene encoding an amino acid sequence represented by:

Sea ID No: 1 Met Thr Asn Leu Gln Thr Phe Glu Leu Pro Thr Glu Val Thr Gly Cys Ala Ala Asp Ile Ser Leu Gly Arg Ala Leu Ile Gln Ala Trp Gln Lys Asp Gly Ile Phe Gln Ile Lys Thr Asp Ser Glu Gln Asp Arg Lys Thr Gln Glu Ala Met Ala Ala Ser Lys Gln Phe Cys Lys Glu Pro Leu Thr Phe Lys Ser Ser Cys Val Ser Asp Leu Thr Tyr Ser Gly Tyr Val Ala Ser Gly Glu Glu Val Thr Ala Gly Lys Pro Asp Phe Pro Glu Ile Phe Thr Val Cys Lys Asp Leu Ser Val Gly Asp Gln Arg Val Lys Ala Gly Trp Pro Cys His Gly Pro Val Pro Trp Pro Asn Asn Thr Tyr Gln Lys Ser Met Lys Thr Phe Met Glu Glu Leu Gly Leu Ala Gly Glu Arg Leu Leu Lys Leu Thr Ala Leu Gly Phe Glu Leu Pro Ile Asn Thr Phe Thr Asp Leu Thr Arg Asp Gly Trp His His Met Arg Val Leu Arg Phe Pro Pro Gln Thr Ser Thr Leu Ser Arg Gly Ile Gly Ala His Thr Asp Tyr Gly Leu Leu Val Ile Ala Ala Gln Asp Asp Val Gly Gly Leu Tyr Ile Arg Pro Pro Val Glu Gly Glu Lys Arg Asn Arg Asn Trp Leu Pro Gly Glu Ser Ser Ala Gly Met Phe Glu His Asp Glu Pro Trp Thr Phe Val Thr Pro Tbr Pro Gly Val Trp Tbr Val Phe Pro Gly Asp Ile Leu Gln Phe Met Thr Gly Gly Gln Leu Leu Ser Thr Pro His Lys Val Lys Leu Asn Thr Arg Glu Arg Phe Ala Cys Ala Tyr Phe His Glu Pro Asn Phe Glu Ala Ser Ala Tyr Pro Leu Phe Glu Pro Ser Ala Asn Glu Arg Ile His Tyr Gly Glu His Phe Thr Asn Met Phe Met Arg Cys Tyr Pro Asp Arg Ile Thr Thr Gln Arg Ile Asn Lys Glu Asn Arg Leu Ala His Leu Glu Asp Leu Lys Lys Tyr Ser Asp Thr Arg Ala Thr Gly Ser

is integrated into bacteria.

DNA encoding the ethylene-forming enzyme of the present invention can be obtained, for example, by the procedures comprising:

preparing a probe with an amino acid sequence coding the ethylene-forming enzyme from *Pseudomonas syringae*; hybridizing this probe with an indigenous plasmid by Southern method to find that a gene for the ethylene-forming enzyme is encoded in the indigenous plasmid DNA; constructing a gene library after transforming *Escherichia coli* (*E. coli*) using restriction enzyme Hind III fragments of this indigenous plasmid DNA and pUC19 as a vector; and screening a positive clone *E. coli* JM109 (pEFE01) from the library by a hybridization using Southern method.

Hind III fragments of about 2.5 kbp from *Pseudononas syringae* were inserted into the plasmid pUC19 in the cell of this positive clone *E. coli* JMO109(pEFE01), and an ethylene-forming activity was found in this positive clone *E. coli* JM109(pEFE01). This positive clone *E. coli* JM109(pEFE01) also expressed a protein that could not be distinguished from the ethylene-forming enzyme protein of *Pseudomonas syringae* as detected by Western blotting method using an antibody for the ethylene forming enzyme.

Deletion mutant strains having various sizes of plasmids were prepared by digesting the inserted 2.5kbp Hind III fragments. The smallest pEFEO1 derivative was obtained from the mutants keeping ethylene-forming activity. The size of the smallest fragment was about 1.5 kbp.

BRIEF DESCRIPTION OF DRAWOMGS

FIG. **1** shows a base sequence of the gene of the ethylene-forming enzyme after cloning and the first half portion of the amino acid sequence translated therefrom.

FIG. **1** shows a base sequence of the gene of the ethylene-forming enzyme after cloning and the latter half portion of the amino acid sequence translated therefrom.

BEST MODE FOR CARRYING OUT THE INVENTION

EXAMPLES

Chromosomal DNA of *Pseudomonas syringae* was extracted with phenol after lysis of cell wall with lysozyme and sodium dodecyl gulfate (SDS), wherein proteins were removed by denaturation and DNA was recovered from the supernatant by ethanol precipitation. The plasmid DNA of *Pseudomonas syringae* wae was extracted by an alkali extraction method.

Extracted DNA was digested with a restriction enzyme Hind III and the digested fragments were subjected to electrophoresis using 0.7% of agarose gel. DNA in the gel after the electrophoresis were transferred to a Nylon membrane by a capillary method, bound onto the membrane by a dry-heat treatment and were subjected to Southern hybridization.

Since the structural gene of the ethylene-forming enzyme (EFE) seemed to be located on the plasmid after the hybridization, the plasmid DNA fragments digested with Hind III are ligated with the *E. coli* vector (pUC19) subjected to an alkali phosphatase treatment, thereby obtaining the chimera plasmid. This chimera plasmid was mixed with competent cells of *Escherichia coli* (*E. coli* YM109) prepared by a calcium chloride method and, after allowing them to stand in an ice bath for an hour, the chimera plasmid was incorporated into the cell with a heat shock, followed by centrifugation, thereby transforming *E. coli* JM108. This *E. coli* JM109 after the transformation was seeded in 2xYT culture medium consisting of 16g/liter of Bactotrypton, 10g/liter of Bactoyeast extract and 5g/liter of sodium chloride. After the cultivation for 1.5 hours, *E. coii* JM109 was separated by centrifugation and inoculated in a selected culture medium.

Colonies having the chimera plasmid expressed in the selected culture medium were screened and these colonies were cultivated in LB culture medium consisting of 10g/liter of Bactotrypton, 5g/liter of Bactoyeast extract and 10g/liter of sodium chloride supplemented with 5 μg/ml of thiamine hydrochloride and 50 μg/ml of ampicillin sodium salt. The plasmid DNA (chimera pUC19) was extracted with an aqueous alkaline, purified by removing RNA and, after treating with Hind III, it was subjected to electrophoresis using 1% of agarose gel. The DNA after electrophoresis was denatured with an aqueous alkaline solution followed by neutralization and blotted on a nylon membrane, and then *E. coli* which retained the gene of the ethylene-forming enzyme was screened by hybridization.

When *E. coli* JM109(pEFE01) was cultivated in the above described LB culture medium supplemented with 5g/ml of L-glutamic acid, 5 μg/ml of thiamine hydrochloride and 50 μg/ml of ampicillin sodium salt, the ethylene-forming activity was 230nl/ml of culture medium/hr. In the above described *E. coli* JM109 (a disclosed strain well known in the art) used as a host, on the other hand, any ethylene-forming activity was not detected and its ethylene-forming rate was On1/ml of culture medium/hr. The term "ethylene-forming activity" used herein refers to an in vitro ethylene-forming ability in a cell-free extract prepared from the cells, and the term "ethylene-forming rate" refers to an in vivo ethylene-forming ability in the culture medium. These characteristics could be detected by conventional methods described, for example, in the above described reference (Nagahama, Fukuda et al., J. Gen. Microbiol., 37, 2281, 1991).

The transformant *Escherichia coli* JM109(pEFE01) used in this invention was deposited to National Institute of Bioscience and Human-Technology, 1–3, Higashi -chome, Tsukuba City, Japan, under the registration number of FERM P-1361 dated Sep. 16, 1992.

It was made clear by Western blotting that an identical protein with the ethylene-forming enzyme of *Pseudomonas syringae* was also present in *E. coli* JM109(pEFE01). Deletion mutant having various size of plasmids were prepared by digesting the inserted 2.5kbp Hind III fragment from its terminal. The smallest pEFE01 transformant retaining the ethylene-forming activity was about 1.5 kbp. In addition, neither site for Hind III nor sites for other restriction enzymes PstI, Dra I, EcoR I and BamH I were present in this fragment.

The base sequence and amino acid sequence of the gene of the ethylene forming-enzyme incorporated in 2.5 kbp Hind III fragment as determined by a dideoxy method are shown in FIG. **1** and FIG. **2**.

Industrial Applicability

According to this invention, bacteria having high ethylene-forming activity can be obtained by transforming bacteria such as *E. coli* which is safe and easy in manipulation.

Moreover, ethylene can be produced with high efficiency by utilizing these transformed bacteria.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i i i) NUMBER OF SEQUENCES: 4

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 350 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular

(i i) MOLECULE TYPE: DNA (genomic)

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met Thr Asn Leu Gln Thr Phe Glu Leu Pro Thr Glu Val Thr Gly Cys

-continued

1				5				10				15			
Ala	Ala	Asp	Ilc	Scr	Lcu	Gly	Arg	Ala	Lcu	Ilc	Gln	Ala	Trp	Gln	Lys
			20					25					30		
Asp	Gly	Ilc	Phc	Gln	Ilc	Lys	Thr	Asp	Scr	Glu	Gln	Asp	Arg	Lys	Thr
		35					40					45			
Gln	Glu	Ala	Mct	Ala	Ala	Scr	Lys	Gln	Phc	Cys	Lys	Glu	Pro	Lcu	Thr
	50					55					60				
Phc	Lys	Scr	Scr	Cys	Val	Scr	Asp	Lcu	Thr	Tyr	Scr	Gly	Tyr	Val	Ala
65					70					75					80
Scr	Gly	Glu	Glu	Val	Thr	Ala	Gly	Lys	Pro	Asp	Phc	Pro	Glu	Ilc	Phc
				85					90					95	
Thr	Val	Cys	Lys	Asp	Lcu	Scr	Val	Gly	Asp	Gln	Arg	Val	Lys	Ala	Gly
			100					105					110		
Trp	Pro	Cys	His	Gly	Pro	Val	Pro	Trp	Pro	Asn	Asn	Thr	Tyr	Gln	Lys
		115					120					125			
Scr	Mct	Lys	Thr	Phc	Mct	Glu	Glu	Lcu	Gly	Lcu	Ala	Gly	Glu	Arg	Lcu
	130					135					140				
Lcu	Lys	Lcu	Thr	Ala	Lcu	Gly	Phc	Glu	Lcu	Pro	Ilc	Asn	Thr	Phc	Thr
145					150					155					160
Asp	Lcu	Thr	Arg	Asp	Gly	Trp	His	His	Mct	Arg	Val	Lcu	Arg	Phc	Pro
				165					170					175	
Pro	Gln	Thr	Scr	Thr	Lcu	Scr	Arg	Gly	Ilc	Gly	Ala	His	Thr	Asp	Tyr
			180					185					190		
Gly	Lcu	Lcu	Val	Ilc	Ala	Ala	Gln	Asp	Asp	Val	Gly	Gly	Lcu	Tyr	Ilc
		195					200					205			
Arg	Pro	Pro	Val	Glu	Gly	Glu	Lys	Arg	Asn	Arg	Asn	Trp	Lcu	Pro	Gly
	210					215					220				
Glu	Scr	Scr	Ala	Gly	Mct	Phc	Glu	His	Asp	Glu	Pro	Trp	Thr	Phc	Val
225					230					235					240
Thr	Pro	Thr	Pro	Gly	Val	Trp	Thr	Val	Phc	Pro	Gly	Asp	Ilc	Lcu	Gln
				245					250					255	
Phc	Mct	Thr	Gly	Gly	Gln	Lcu	Lcu	Scr	Thr	Pro	His	Lys	Val	Lys	Lcu
			260					265					270		
Asn	Thr	Arg	Glu	Arg	Phc	Ala	Cys	Ala	Tyr	Phc	His	Glu	Pro	Asn	Phc
		275					280					285			
Glu	Ala	Scr	Ala	Tyr	Pro	Lcu	Phc	Glu	Pro	Scr	Ala	Asn	Glu	Arg	Ilc
	290					295					300				
His	Tyr	Gly	Glu	His	Phc	Thr	Asn	Mct	Phc	Mct	Arg	Cys	Tyr	Pro	Asp
305					310					315					320
Arg	Ilc	Thr	Thr	Gln	Arg	Ilc	Asn	Lys	Glu	Asn	Arg	Lcu	Ala	His	Lcu
				325					330					335	
Glu	Asp	Lcu	Lys	Lys	Tyr	Scr	Asp	Thr	Arg	Ala	Thr	Gly	Scr		
				340				345				350			

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1050 basc pairs
(B) TYPE: nuclcic acid
(C) STRANDEDNESS: doublc
(D) TOPOLOGY: circular

(i i) MOLECULE TYPE: DNA (genomic)

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ATGACCAACC TACAGACTTT CGAGTTGCCT ACCGAGGTAA CCGGCTGCGC CGCCGATATC 60

-continued

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TCATTGGGAA GGGCGCTGAT CCAAGCCTGG CAAAAAGATG GCATTTTTCA GATCAAGACC    120
GATAGTGAGC AGGATCGCAA AACGCAGGAA GCAATGGCTG CTAGCAAGCA GTTTTGCAAG    180
GAACCGCTGA CTTTTAAGAG TAGCTGCGTT AGCGATCTGA CCTACAGCGG CTATGTTGCG    240
TCAGGCGAGG AAGTCACAGC TGGTAAACCT GATTTCCCTG AAATCTTCAC TGTCTGCAAG    300
GACTTGTCGG TAGGCGATCA GCGTGTAAAA GCCGGCTGGC CTTGCCATGG TCCGGTGCCA    360
TGGCCAAATA ACACCTATCA GAAAAGCATG AAGACCTTCA TGGAAGAGCT GGGTTTAGCG    420
GGCGAACGGT TGCTCAAACT GACAGCGCTC GGCTTTGAAC TACCCATCAA CACGTTCACC    480
GACTTAACTC GCGATGGTTG GCACCACATG CGTGTATTAC GCTTCCCGCC CCAAACATCC    540
ACGCTGTCCC GTGGAATTGG TGCGCACACT GACTATGGGT TGTTGGTAAT TGCCGCTCAG    600
GACGATGTTG GTGGCTTATA TATTCGCCCT CCAGTCGAGG GAGAGAAGCG TAATCGTAAC    660
TGGTTGCCTG GTGAGAGCTC AGCAGGCATG TTTGAGCACG ATGAACCTTG GACCTTCGTG    720
ACGCCCACCC CAGGCGTGTG GACAGTTTTC CCAGGTGATA TCTTGCAGTT CATGACCGGC    780
GGCCAGCTGC TTTCCACTCC GCACAAGGTT AAGCTCAATA CCCGCGAACG TTTCGCCTGC    840
GCTTATTTTC ATGAGCCTAA TTTTGAAGCA TCCGCCTATC CGTTGTTCGA GCCCAGCGCC    900
AATGAGCGTA TTCATTATGG TGAGCACTTT ACCAACATGT TTATGCGTTG CTATCCAGAT    960
CGGATCACCA CCCAGAGGAT CAACAAGGAG AATCGCCTGG CGCACTTGGA GGACTTGAAG   1020
AAGTATTCGG ACACCCGCGC GACAGGCTCA                                    1050
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1809 basc pairs
(B) TYPE: nuclcic acid
(C) STRANDEDNESS: doublc
(D) TOPOLOGY: circular

(i i) MOLECULE TYPE: DNA (gcnomic)

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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GCGACCGCGT GTCAGCAACG ACAATCCTTA CTCGGAGTCG CTGTTCAGGA CACTGAAGTA     60
CTGCCCGCAA TGGCCGCAGG ATGGGTTTGC CAGTCTTGAC GCGGCACGCT CTGGGTGAGG    120
GATTTCATGC GTTGGTACAA CAATGATCAC CGGCACAGCC GAATCCGCTT CGTGACACCG    180
GCTGAGCGGC ATCGAGGGCT GGATCATCAG ATCCTGGCCA AGCGACATGA GCTGTACGAG    240
CTAGCCAGAG AGAAAAGGCC GGAGCGGTGG TCGAGGGAGA CACGCAACTG GGAACCGATC    300
GGCACCGTGC TGTTAAACCC GGATCGAGAG CAAGACGTTG AGAAAAAAGC AGCATAGTTA    360
GACGGTTGAC GCGACAACTA CCTTGAAAAA CGCCGGCAGG GACGCTCATG ATTCATGCTC    420
CAAGCAGGTG GGGTGTTTTT CCGTCGCTTG GACTTTGTTC TCCCGACGTC GTTTGGAATG    480
AGCATCCGTC CCTTTATATG GATAAAGAAG AGACTAGCAT GACCAACCTA CAGACTTTCG    540
AGTTGCCTAC CGAGGTAACC GGCTGCGCCG CCGATATCTC ATTGGGAAGG GCGCTGATCC    600
AAGCCTGGCA AAAAGATGGC ATTTTTCAGA TCAAGACCGA TAGTGAGCAG GATCGCAAAA    660
CGCAGGAAGC AATGGCTGCT AGCAAGCAGT TTTGCAAGGA ACCGCTGACT TTTAAGAGTA    720
GCTGCGTTAG CGATCTGACC TACAGCGGCT ATGTTGCGTC AGGCGAGGAA GTCACAGCTG    780
GTAAACCTGA TTTCCCTGAA ATCTTCACTG TCTGCAAGGA CTTGTCGGTA GGCGATCAGC    840
GTGTAAAAGC CGGCTGGCCT TGCCATGGTC CGGTGCCATG GCCAAATAAC ACCTATCAGA    900
AAAGCATGAA GACCTTCATG GAAGAGCTGG GTTTAGCGGG CGAACGGTTG CTCAAACTGA    960
CAGCGCTCGG CTTTGAACTA CCCATCAACA CGTTCACCGA CTTAACTCGC GATGGTTGGC   1020
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ACCACATGCG TGTATTACGC TTCCCGCCCC AAACATCCAC GCTGTCCCGT GGAATTGGTG    1080
CGCACACTGA CTATGGGTTG TTGGTAATTG CCGCTCAGGA CGATGTTGGT GGCTTATATA    1140
TTCGCCCTCC AGTCGAGGGA GAGAAGCGTA ATCGTAACTG GTTGCCTGGT GAGAGCTCAG    1200
CAGGCATGTT TGAGCACGAT GAACCTTGGA CCTTCGTGAC GCCCACCCCA GGCGTGTGGA    1260
CAGTTTTCCC AGGTGATATC TTGCAGTTCA TGACCGGCGG CCAGCTGCTT TCCACTCCGC    1320
ACAAGGTTAA GCTCAATACC CGCGAACGTT TCGCCTGCGC TTATTTTCAT GAGCCTAATT    1380
TTGAAGCATC CGCCTATCCG TTGTTCGAGC CCAGCGCCAA TGAGCGTATT CATTATGGTG    1440
AGCACTTTAC CAACATGTTT ATGCGTTGCT ATCCAGATCG GATCACCACC CAGAGGATCA    1500
ACAAGGAGAA TCGCCTGGCG CACTTGGAGG ACTTGAAGAA GTATTCGGAC ACCCGCGCGA    1560
CAGGCTCATG AGTCGACACC CTGCCCGGTG CTGCCGGACA GGGGCCTTAT CGTTACTGGT    1620
GACTAATAAT TGGCATATCA ATGTCCACTC AGCACCCAGA TCTTATGATC TGGGTGCTGA    1680
GTGGAGCAAT GTAACCATTA TGCTGAGCGT TCATGCATAG GAATTTCAAT AATTCCTATA    1740
CAAGGCAATC CGCCGAAAAA GGTCCCCTCG GCGTGATGCC AACGTGGCGT CGATGTCGGC    1800
AAAAAGCTT                                                            1809
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1809 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular

(i i) MOLECULE TYPE: DNA (genomic)

(i x) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 519..1568

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
GCGACCGCGT GTCAGCAACG ACAATCCTTA CTCGGAGTCG CTGTTCAGGA CACTGAAGTA      60
CTGCCCGCAA TGGCCGCAGG ATGGGTTTGC CAGTCTTGAC GCGGCACGCT CTGGGTGAGG     120
GATTTCATGC GTTGGTACAA CAATGATCAC CGGCACAGCC GAATCCGCTT CGTGACACCG     180
GCTGAGCGGC ATCGAGGGCT GGATCATCAG ATCCTGGCCA AGCGACATGA GCTGTACGAG     240
CTAGCCAGAG AGAAAAGGCC GGAGCGGTGG TCGAGGGAGA CACGCAACTG GGAACCGATC     300
GGCACCGTGC TGTTAAACCC GGATCGAGAG CAAGACGTTG AGAAAAAAGC AGCATAGTTA     360
GACGGTTGAC GCGACAACTA CCTTGAAAAA CGCCGGCAGG GACGCTCATG ATTCATGCTC     420
CAAGCAGGTG GGGTGTTTTT CCGTCGCTTG GACTTTGTTC TCCCGACGTC GTTTGGAATG     480
AGCATCCGTC CCTTTATATG GATAAAGAAG AGACTAGC ATG ACC AAC CTA CAG          533
                                    Met Thr Asn Leu Gln
                                     1               5

ACT TTC GAG TTG CCT ACC GAG GTA ACC GGC TGC GCC GCC GAT ATC TCA      581
Thr Phe Glu Leu Pro Thr Glu Val Thr Gly Cys Ala Ala Asp Ile Ser
            10                  15                  20

TTG GGA AGG GCG CTG ATC CAA GCC TGG CAA AAA GAT GGC ATT TTT CAG      629
Leu Gly Arg Ala Leu Ile Gln Ala Trp Gln Lys Asp Gly Ile Phe Gln
        25                  30                  35

ATC AAG ACC GAT AGT GAG CAG GAT CGC AAA ACG CAG GAA GCA ATG GCT      677
Ile Lys Thr Asp Ser Glu Gln Asp Arg Lys Thr Gln Glu Ala Met Ala
    40                  45                  50

GCT AGC AAG CAG TTT TGC AAG GAA CCG CTG ACT TTT AAG AGT AGC TGC      725
Ala Ser Lys Gln Phe Cys Lys Glu Pro Leu Thr Phe Lys Ser Ser Cys
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      55                  60                  65

GTT AGC GAT CTG ACC TAC AGC GGC TAT GTT GCG TCA GGC GAG GAA GTC          773
Val Ser Asp Leu Thr Tyr Ser Gly Tyr Val Ala Ser Gly Glu Glu Val
 70                  75                  80                  85

ACA GCT GGT AAA CCT GAT TTC CCT GAA ATC TTC ACT GTC TGC AAG GAC          821
Thr Ala Gly Lys Pro Asp Phe Pro Glu Ile Phe Thr Val Cys Lys Asp
                 90                  95                 100

TTG TCG GTA GGC GAT CAG CGT GTA AAA GCC GGC TGG CCT TGC CAT GGT          869
Leu Ser Val Gly Asp Gln Arg Val Lys Ala Gly Trp Pro Cys His Gly
            105                 110                 115

CCG GTG CCA TGG CCA AAT AAC ACC TAT CAG AAA AGC ATG AAG ACC TTC          917
Pro Val Pro Trp Pro Asn Asn Thr Tyr Gln Lys Ser Met Lys Thr Phe
        120                 125                 130

ATG GAA GAG CTG GGT TTA GCG GGC GAA CGG TTG CTC AAA CTG ACA GCG          965
Met Glu Glu Leu Gly Leu Ala Gly Glu Arg Leu Leu Lys Leu Thr Ala
    135                 140                 145

CTC GGC TTT GAA CTA CCC ATC AAC ACG TTC ACC GAC TTA ACT CGC GAT         1013
Leu Gly Phe Glu Leu Pro Ile Asn Thr Phe Thr Asp Leu Thr Arg Asp
150                 155                 160                 165

GGT TGG CAC CAC ATG CGT GTA TTA CGC TTC CCG CCC CAA ACA TCC ACG         1061
Gly Trp His His Met Arg Val Leu Arg Phe Pro Pro Gln Thr Ser Thr
                170                 175                 180

CTG TCC CGT GGA ATT GGT GCG CAC ACT GAC TAT GGG TTG TTG GTA ATT         1109
Leu Ser Arg Gly Ile Gly Ala His Thr Asp Tyr Gly Leu Leu Val Ile
            185                 190                 195

GCC GCT CAG GAC GAT GTT GGT GGC TTA TAT ATT CGC CCT CCA GTC GAG         1157
Ala Ala Gln Asp Asp Val Gly Gly Leu Tyr Ile Arg Pro Pro Val Glu
        200                 205                 210

GGA GAG AAG CGT AAT CGT AAC TGG TTG CCT GGT GAG AGC TCA GCA GGC         1205
Gly Glu Lys Arg Asn Arg Asn Trp Leu Pro Gly Glu Ser Ser Ala Gly
    215                 220                 225

ATG TTT GAG CAC GAT GAA CCT TGG ACC TTC GTG ACG CCC ACC CCA GGC         1253
Met Phe Glu His Asp Glu Pro Trp Thr Phe Val Thr Pro Thr Pro Gly
230                 235                 240                 245

GTG TGG ACA GTT TTC CCA GGT GAT ATC TTG CAG TTC ATG ACC GGC GGC         1301
Val Trp Thr Val Phe Pro Gly Asp Ile Leu Gln Phe Met Thr Gly Gly
                250                 255                 260

CAG CTG CTT TCC ACT CCG CAC AAG GTT AAG CTC AAT ACC CGC GAA CGT         1349
Gln Leu Leu Ser Thr Pro His Lys Val Lys Leu Asn Thr Arg Glu Arg
            265                 270                 275

TTC GCC TGC GCT TAT TTT CAT GAG CCT AAT TTT GAA GCA TCC GCC TAT         1397
Phe Ala Cys Ala Tyr Phe His Glu Pro Asn Phe Glu Ala Ser Ala Tyr
        280                 285                 290

CCG TTG TTC GAG CCC AGC GCC AAT GAG CGT ATT CAT TAT GGT GAG CAC         1445
Pro Leu Phe Glu Pro Ser Ala Asn Glu Arg Ile His Tyr Gly Glu His
    295                 300                 305

TTT ACC AAC ATG TTT ATG CGT TGC TAT CCA GAT CGG ATC ACC ACC CAG         1493
Phe Thr Asn Met Phe Met Arg Cys Tyr Pro Asp Arg Ile Thr Thr Gln
310                 315                 320                 325

AGG ATC AAC AAG GAG AAT CGC CTG GCG CAC TTG GAG GAC TTG AAG AAG         1541
Arg Ile Asn Lys Glu Asn Arg Leu Ala His Leu Glu Asp Leu Lys Lys
                330                 335                 340

TAT TCG GAC ACC CGC GCG ACA GGC TCA TGAGTCGACA CCCTGCCCGG               1588
Tyr Ser Asp Thr Arg Ala Thr Gly Ser
            345                 350

TGCTGCCGGA CAGGGGCCTT ATCGTTACTG GTGACTAATA ATTGGCATAT CAATGTCCAC       1648

TCAGCACCCA GATCTTATGA TCTGGGTGCT GAGTGGAGCA ATGTAACCAT TATGCTGAGC       1708

GTTCATGCAT AGGAATTTCA ATAATTCCTA TACAAGGCAA TCCGCCGAAA AAGGTCCCCT       1768
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CGGCGTGATG CCAACGTGGC GTCGATGTCG GCAAAAAGCT T	1809

What is claimed is:

1. A DNA fragment comprising a gene encoding an ethylene-forming enzyme of bacteria represented by the following amino acid sequence:

SEQ ID NO:1:

Met Thr Asn Leu Gln Thr Phe Glu Leu Pro Thr Glu Val
Thr Gly Cys Ala Ala Asp Ile Ser Leu Gly Arg Ala
Leu Ile Gln Ala Trp Gln Lys Asp Gly Ile Phe Gln Ile
Lys Thr Asp Ser Glu Gln Asp Arg Lys Thr Gln Glu
Ala Met Ala Ala Ser Lys Gln Phe Cys Lys Glu Pro
Leu Thr Phe Lys Ser Ser Cys Val Ser Asp Leu Thr
Tyr Ser Gly Tyr Val Ala Ser Gly Glu Glu Val Thr Ala
Gly Lys Pro Asp Phe Pro Glu Ile Phe Thr Val Cys Lys
Asp Leu Ser Val Gly Asp Gln Arg Val Lys Ala Gly
Trp Pro Cys His Gly Pro Val Pro Trp Pro Asn Asn
Thr Tyr Gln Lys Ser Met Lys Thr Phe Met Glu Glu
Leu Gly Leu Ala Gly Glu Arg Leu Leu Lys Leu Thr
Ala Leu Gly Phe Glu Leu Pro Ile Asn Thr Phe Thr
Asp Leu Thr Arg Asp Gly Trp His His Met Arg Val
Leu Arg Phe Pro Pro Gln Thr Ser Thr Leu Ser Arg
Gly Ile Gly Ala His Thr Asp Tyr Gly Leu Leu Val Ile
Ala Ala Gln Asp Asp Val Gly Gly Leu Tyr Ile Arg Pro
Pro Val Glu Gly Glu Lys Arg Asn Arg Asn Trp Leu
Pro Gly Glu Ser Ser Ala Gly Met Phe Glu His Asp
Glu Pro Trp Thr Phe Val Thr Pro Thr Pro Gly Val Trp
Thr Val Phe Pro Gly Asp Ile Leu Gln Phe Met Thr
Gly Gly Gln Leu Leu Ser Thr Pro His Lys Val Lys
Leu Asn Thr Arg Glu Arg Phe Ala Cys Ala Tyr Phe
His Glu Pro Asn Phe Glu Ala Ser Ala Tyr Pro Leu
Phe Glu Pro Ser Ala Asn Glu Arg Ile His Tyr Gly Glu
His Phe Thr Asn Met Phe Met Arg Cys Tyr Pro Asp
Arg Ile Thr Thr Gln Arg Ile Asn Lys Glu Asn Arg Leu
Ala His Leu Glu Asp Leu Lys Lys Tyr Ser Asp Thr
Arg Ala Thr Gly Ser

2. A DNA fragment according to claim **1** which comprises a gene encoding an ethylene-forming enzyme of bacteria, wherein said bacteria belong to a genus Pseudomonas.

3. A DNA fragment according to claim **1** which comprises a gene encoding an ethylene-forming enzyme of bacteria represented by the following base sequence:

SEQ ID NO:2

ATG ACC AAC CTA CAG ACT TTC GAG TTG CCT
ACC GAG GTA ACC GGC TGC GCC GCC GAT
ATC TCA TTG GGA AGG GCG CTG ATC CAA
GCC TGG CAA AAA GAT GGC ATT TTT CAG
ATC AAG ACC GAT AGT GAG CAG GAT CGC
AAA ACG CAG GAA GCA ATG GCT GCT AGC
AAG CAG TTT TGC AAG GAA CCG CTG ACT
TTT AAG AGT AGC TGC GTT AGC GAT CTG
ACC TAC AGC GGC TAT GTT GCG TCA GGC
GAG GAA GTC ACA GCT GGT AAA CCT GAT
TTC CCT GAA ATC TTC ACT GTC TGC AAG
GAC TTG TCG GTA GGC GAT CAG CGT GTA
AAA GCC GGC TGG CCT TGC CAT GGT CCG
GTG CCA TGG CCA AAT AAC ACC TAT CAG
AAA AGC ATG AAG ACC TTC ATG GAA GAG
CTG GGT TTA GCG GGC GAA CGG TTG CTC
AAA CTG ACA GCG CTC GGC TTT GAA CTA
CCC ATC AAC ACG TTC ACC GAC TTA ACT
CGC GAT GGT TGG CAC CAC ATG CGT GTA
TTA CGC TTC CCG CCC CAA ACA TCC ACG
CTG TCC CGT GGA ATT GGT GCG CAC ACT
GAC TAT GGG TTG TTG GTA ATT GCC GCT
CAG GAC GAT GTT GGT GGC TTA TAT ATT
CGC CCT CCA GTC GAG GGA GAG AAG CGT
AAT CGT AAC TGG TTG CCT GGT GAG AGC
TCA GCA GGC ATG TTT GAG CAC GAT GAA
CCT TGG ACC TTC GTG ACG CCC ACC CCA
GGC GTG TGG ACA GTT TTC CCA GGT GAT
ATC TTG CAG TTC ATG ACC GGC GGC CAG
CTG CTT TCC ACT CCG CAC AAG GTT AAG
CTC AAT ACC CGC GAA CGT TTC GCC TGC
GCT TAT TTT CAT GAG CCT AAT TTT GAA
GCA TCC GCC TAT CCG TTG TTC GAG CCC
AGC GCC AAT GAG CGT ATT CAT TAT GGT
GAG CAC TTT ACC AAC ATG TTT ATG CGT
TGC TAT CCA GAT CGG ATC ACC ACC CAG
AGG ATC AAC AAG GAG AAT CGC CTG GCG
CAC TTG GAG GAC TTG AAG AAG TAT TCG
GAC ACC CGC GCG ACA GGC TCA

4. A DNA fragment according to claim **1** which comprises a gene encoding an ethylene-forming enzyme of bacteria, wherein said fragment has a base sequence of at least from No. 519 to No. 1568 of the following base sequence:

SEQ ID NO:3:

```
          10         20         30         40         50
GCGACCGCGTGTCAGCAACGACAATCCTTACTCGGAGTCGCTGTTCAGGACACTGAAGT

          60         70         80         90        100        110
ACTGCCCGCAATGGCCGCAGGATGGGTTTGCCAGTCTTGACGCGGCACGCTCTGGGTGAG

         120        130        140        150        160        170
GGATTTCATGCGTTGGTACAACAATGATCACCGGCACAGCCGAATCCGCTTCGTGACACC

         180        190        200        210        220        230
GGCTGAGCGGCATCGAGGGCTGGATCATCAGATCCTGGCCAAGCGACATGAGCTGTACGA

         240        250        260        270        280        290
GCTAGCCAGAGAGAAAAGGCCGGAGCGGTGGTCGAGGGAGACACGCAACTGGGAACCGAT

         300        310        320        330        340        350
CGGCACCGTGCTGTTAAACCCGGATCGAGAGCAAGACGTTGAGAAAAAAGCAGCATAGTT

         360        370        380        390        400        410
AGACGGTTGACGCGACAACTACCTTGAAAAACGCCGGCAGGGACGCTCATGATTCATGCT
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420 430 440 450 460 470
CCAAGCAGGTGGGGTGTTTTTCCGTCGCTTGGACTTTGTTCTCCCGACGTCGTTTGGAAT

480 490 500 510 520 530
GAGCATCCGTCCCTTTATATGGATAAAGAAGAGACTAGCATGACCAACCTACAGACTTTC

540 550 560 570 580 590
GAGTTGCCTACCGAGGTAACCGGCTGCGCCGCCGATATCTCATTGGGAAGGGCGCTGATC

600 610 620 630 640 650
CAAGCCTGGCAAAAAGATGGCATTTTTCAGATCAAGACCGATAGTGAGCAGGATCGCAAA

660 670 680 690 700 710
ACGCAGGAAGCAATGGCTGCTAGCAAGCAGTTTTGCAAGGAACCGCTGACTTTTAAGAGT

720 730 740 750 760 770
AGCTGCGTTAGCGATCTGACCTACAGCGGCTATGTTGCGTCAGGCGAGGAAGTCACAGCT

780 790 800 810 820 830
GGTAAACCTGATTTCCCTGAAATCTTCACTGTCTGCAAGGACTTGTCGGTAGGCGATCAG

840 850 860 870 880 890
CGTGTAAAAGCCGGCTGGCCTTGCCGTGGTCCGGTGCCATGGCCAAATAACACCTATCAG

900 910 920 930 940 950
AAAAGCATGAAGACCTTCATGGAAGAGCTGGGTTTAGCGGGCGAACGGTTGCTCAAACTG

960 970 980 990 1000 1010
ACAGCGCTCGGCTTTGAACTACCCATCAACACGTTCACCGACTTAACTCGCGATGGTTGG

1020 1030 1040 1050 1060 1070
CACCACATGCGTGTATTACGCTTCCCGCCCCAAACATCCACGCTGTCCCGTGGAATTGGT

1080 1090 1100 1110 1120 1130
GCGCACACTGACTATGGGTTGTTGGTAATTGCCGCTCAGGACGATGTTGGTGGCTTATAT

1140 1150 1160 1170 1180 1190
ATTCGCCCTCCAGTCGAGGGAGAGAAGCGTAATCGTAACTGGTTGCCTGGTGAGAGCTCA

1200 1210 1220 1230 1240 1250
GCAGGCATGTTTGAGCACGATGAACCTTGGACCTTCGTGACGCCCACCCCAGGCGTGTGG

1260 1270 1280 1290 1300 1310
ACAGTTTTCCCAGGTGATATCTTGCAGTTCATGACCGGCGGCCAGCTGCTTTCCACTCCG

1320 1330 1340 1350 1360 1370
CACAAGGTTAAGCTCAATACCCGCGAACGTTTCGCCTGCGCTTATTTTCATGAGCCTAAT

1380 1390 1400 1410 1420 1430
TTTGAAGCATCCGCCTATCCGTTGTTCGAGCCCAGCGCCAATGAGCGTATTCATTATGGT

1440 1450 1460 1470 1480 1490
GAGCACTTTACCAACATGTTTATGCGTTGCTATCCAGATCGGATCACCACCCAGAGGATC

1500 1510 1520 1530 1540 1550
AACAAGGAGAATCGCCTGGCGCACTTGGAGGACTTGAAGAAGTATTCGGACACCCGCGCG

1560 1570 1580 1590 1600 1610
ACAGGCTCATGAGTCGACACCCTGCCCGGTGCTGCCGGACAGGGGCCTTATCGTTACTGG

1620 1630 1640 1650 1660 1670
TGACTAATAATTGGCATATCAATGTCCACTCAGCACCCAGATCTTATGATCTGGGTGCTG

1680 1690 1700 1710 1720 1730
AGTGGAGCAATGTAACCATTATGCTGAGCGTTCATGCATAGGAATTTCAATAATTCCTAT

1740 1750 1760 1770 1780 1790
ACAAGGCAATCCGCCGAAAAAGGTCCCCTCGGCGTGATGCCAACGTGGCGTCGATGTCGG

1800
CAAAAAGCTT

5. A vector which includes a DNA fragment which encodes the amino acid sequence defined in SEQ ID NO:1.

6. A host cell which has been transformed by the vector of claim **5**.

7. The host cell of claim **6** wherein said host cell is *E. coli*.

* * * * *